PROJECT REVIEW

"Detection of autologous blood transfusion using activated red blood cells (the red blood cells eNOS system)"

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Transfusing red blood cells (RBCs) is the oldest form of blood doping and - as the very recent findings around cycling teams and further personal confessions of athletes demonstrate – is still or again used widely in top level sports. Autologous blood transfusion – up to now – is not detectable. It requires that the blood is stored for a longer period prior to reinfusion.

Whereas red blood cells were for a long time regarded as mere O2-transport vehicles, there is evidence from recent studies that erythrocytes also possess enzymes that actively synthesize nitric oxide (NOS) and that alterations of NOS activation (translocation and phosphorylation) may be an indicator for autologous erythrocyte manipulation. Furthermore, adhesion receptors like CD47 antigen are present on RBCs and undergo alterations during blood manipulation. RBC NOS and adhesion receptors are detectable with immunohistochemical methods. Both are also be detectable by means of fluorescence activated cell sorting (FACS) after adaptation of the method, guaranteeing analysis of big numbers of blood samples in a short time.

Our research group has demonstrated that the procedure of blood sampling, storage, and re-infusion in patients with hip-endoprothetic surgery induce an increase in RBC NOS activity in the RBC concentrate as well as in the patient's blood after re-infusion. We also found RBC NOS activation during cardiopulmonary bypass. Furthermore, a progressive decrease in adhesion receptors in stored RBC concentrates could be demonstrated. Altogether, these data indicate, that an autologous blood transfusion induces a longer lasting activation of RBCs NOS system as well as changes in adhesion receptor expression. This has a high chance to be used for the detection of autologous blood transfusions when misused in sports. The relevant diagnostic approaches (immunohistochemistry plus gray-scale analysis and fluorescence activated cell sorting (FACS)) to quantify NOS and adhesion receptors of RBCs, therefore, may provide a new effective strategy in the fight against autologous blood doping.

We will investigate 24 healthy male and female physically active subjects prior to 500 ml blood donation, as well as prior to and several days after re-infusion of the autologous blood concentrate after storage of 2 to 4 weeks. RBC NOS activity and adhesion receptors will be analysed in venous blood samples by means of immunohistochemistry and FACS. Furthermore, changes in blood total haemoglobin mass (tHb) will be analysed several times from prior to the donation until 2 weeks after re-infusion by means of optimized CO rebreathing method, allowing a quantification of the percentage of the re-infused amount of blood. Aerobic performance ($V'_{O2}max$) will be tested several times for the analysis of the physiological effects of the procedures.

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Results and Conclusion

Blood donation, but not red-blood cell re-infusion activated red-blood cell nitric oxid synthase (NOS) system at serine 116 and serine 1177 in healthy, moderately trained subjects. Therefore, red-blood cell NOS activation as measured by serine 116 and/or serine 1177 phosphorylation does not seem to be a valuable parameter for the detection of autologous blood doping in sports.

However, there seems to be a physiological correlation between the red-blood cell NOS phosphorylation at serine 116 and the number of reticulocytes in the blood which remains to be elucidated in further studies.